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SUBSTITUTE SEQUENCE LISTING

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TECH CENTER 1600/2900

<110> Eck, Jürgen
Schmidt, Arno
Zinke, Holger

<120> Recombinant Fusion Proteins Based on
Ribosome-Inactivating Proteins of the mistletoe Viscum
album

<130> 09282-5

<140> 09/347,064

<141> 1999-07-02

<150> PCT/EP98/00009

<151> 1998-01-02

<150> EP 97 10 0012.0

<151> 1997-01-02

<160> 38

<170> PatentIn Ver. 2.1

<210> 1

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<213> Viscum album

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gcttaccaag caggcgacca atcctacttt ttgcgcgacg caccacgcgg cgcggaaacg 300
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gtcacggcgc ttcggttttcc gggcggcagc acgcgtaccc aagctcggtc gattttaatc 480
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cggttggcta taccctccgg taacttcgtg acgttgacca atgttcgcca cgtgatcgcc 720
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<210> 2

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<213> Viscum album

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Glu Tyr Phe Arg Phe Ile Thr Leu Leu Arg Asp Tyr Val Ser Ser Gly
20          25          30
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Ser Phe Ser Asn Glu Ile Pro Leu Leu Arg Gln Ser Thr Ile Pro Val
35 40 45

Ser Asp Ala Gln Arg Phe Val Leu Val Glu Leu Thr Asn Gln Gly Gly
50 55 60

Asp Ser Ile Thr Ala Ala Ile Asp Val Thr Asn Leu Tyr Val Val Ala
65 70 75 80

Tyr Gln Ala Gly Asp Gln Ser Tyr Phe Leu Arg Asp Ala Pro Arg Gly
85 90 95

Ala Glu Thr His Leu Phe Thr Gly Thr Thr Arg Ser Ser Leu Pro Phe
100 105 110

Asn Gly Ser Tyr Pro Asp Leu Glu Arg Tyr Ala Gly His Arg Asp Gln
115 120 125

Ile Pro Leu Gly Ile Asp Gln Leu Ile Gln Ser Val Thr Ala Leu Arg
130 135 140

Phe Pro Gly Gly Ser Thr Arg Thr Gln Ala Arg Ser Ile Leu Ile Leu
145 150 155 160

E1. Ile Gln Met Ile Ser Glu Ala Ala Arg Phe Asn Pro Ile Leu Trp Arg
165 170 175

Ala Arg Gln Tyr Ile Asn Ser Gly Ala Ser Phe Leu Pro Asp Val Tyr
180 185 190

Met Leu Glu Leu Glu Thr Ser Trp Gly Gln Gln Ser Thr Gln Val Gln
195 200 205

His Ser Thr Asp Gly Val Phe Asn Asn Pro Ile Arg Leu Ala Ile Pro
210 215 220

Pro Gly Asn Phe Val Thr Leu Thr Asn Val Arg Asp Val Ile Ala Ser
225 230 235 240

Leu Ala Ile Met Leu Phe Val Cys Gly Glu Arg Pro
245 250

<210> 3

<211> 828

<212> DNA

<213> Viscum album

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cagttgtggc cctccaagtc caacaatgat ccgaatcagt tgtggacgat caaaagggat 180
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gtgatgatct tcgactgtaa tactgctgtg cgggaggcca ctctttggca gatatggggc 300
aatgggacca tcatcaatcc aagatccaat ctgggttttg cagcatcatc tggaatcaaa 360
ggcactacgc ttacggtgca aacactggat tacacgttgg gacagggctg gcttgccggt 420

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aatgataccg cccaacgcga ggtgaccata tatgggttca gggacctttg catggaatca 480
aatggaggga gtgtgtgggt ggagacgtgc gtgagtagcc aaaagaacca aagatgggct 540
ttgtacgggg atggttctat acgccccaaa caaaaccaag accaatgcct cacctgtggg 600
agagactccg tttcaacagt aatcaatata gttagctgca gcgctggatc gtctgggcag 660
cgatgggtgt ttaccaatga aggggccatt ttgaatttaa agaatgggtt ggccatggat 720
gtggcgcaag caaatccaaa gctccgccga ataatcatct atcctgccac agggaaaacca 780
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<210> 4

<211> 267

<212> PRT

<213> Viscum album

<400> 4

Asp Asp Val Thr Cys Ser Ala Ser Glu Pro Thr Val Arg Ile Val Gly
1 5 10 15

Arg Asn Gly Met Cys Val Asp Val Arg Asp Asp Asp Phe Arg Asp Gly
20 25 30

Asn Gln Ile Gln Leu Trp Pro Ser Lys Ser Asn Asn Asp Pro Asn Gln
35 40 45

Leu Trp Thr Ile Lys Arg Asp Gly Thr Ile Arg Ser Asn Gly Ser Cys
50 55 60

Leu Thr Thr Tyr Gly Tyr Thr Ala Gly Val Tyr Val Met Ile Phe Asp
65 70 75 80

Cys Asn Thr Ala Val Arg Glu Ala Thr Leu Trp Gln Ile Trp Gly Asn
85 90 95

Gly Thr Ile Ile Asn Pro Arg Ser Asn Leu Val Leu Ala Ala Ser Ser
100 105 110

Gly Ile Lys Gly Thr Thr Leu Thr Val Gln Thr Leu Asp Tyr Thr Leu
115 120 125

Gly Gln Gly Trp Leu Ala Gly Asn Asp Thr Ala Pro Arg Glu Val Thr
130 135 140

Ile Tyr Gly Phe Arg Asp Leu Cys Met Glu Ser Asn Gly Gly Ser Val
145 150 155 160

Trp Val Glu Thr Cys Val Ser Ser Gln Lys Asn Gln Arg Trp Ala Leu
165 170 175

Tyr Gly Asp Gly Ser Ile Arg Pro Lys Gln Asn Gln Asp Gln Cys Leu
180 185 190

Thr Cys Gly Arg Asp Ser Val Ser Thr Val Ile Asn Ile Val Ser Cys
195 200 205

Ser Ala Gly Ser Ser Gly Gln Arg Trp Val Phe Thr Asn Glu Gly Ala
210 215 220

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Ile Leu Asn Leu Lys Asn Gly Leu Ala Met Asp Val Ala Gln Ala Asn
225 230 235 240

Pro Lys Leu Arg Arg Ile Ile Ile Tyr Pro Ala Thr Gly Lys Pro Asn
245 250 255

Gln Met Trp Leu Pro Val Pro Gly Gly Tyr His
260 265

<210> 5
<211> 72
<212> DNA
<213> Viscum album

<400> 5
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gatgttacat gt 72

<210> 6
<211> 17
<212> PRT
<213> Viscum album

E1
<400> 6
Ser Ser Ser Glu Val Arg Tyr Trp Pro Leu Val Ile Arg Pro Val Ile
1 5 10 15
Ala

<210> 7
<211> 756
<212> DNA
<213> Viscum album

<400> 7
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atcacgcttc tccgagatta tgtctcaagc ggaagctttt ccaatgagat accactcttg 120
cgtcagtcta cgatccccgt ctccgatgcg caaagatttg tcttgggtgga gctcaccaac 180
cagggggggag actcgatcac ggccgccatc gacggtacca atctgtacgt cgtgggttac 240
caagcaggcg accaatccta ctttttgcg cagcaccac gcggcgcgga aacgcatttc 300
ttcaccggca ccaccgatc ctctctcca ttcaacggaa gctaccctga tctggagcga 360
tacgccggac atagggacca gatccctctc ggtatagacc aactcattca atccgtcacg 420
gcgcttcggt ttccgggcg cagcacgcgt acccaagctc gttcgatttt aatcctcatt 480
cagatgatct ccgaggccgc cagattcaat cccatcttat ggagggtcgc ccaatacatt 540
aacagtgggg cgtcatttct gccagacgtg tacatgctgg agctggagac gagttggggc 600
caacaatcca cgcaagtcca gcattcaacc gatggcggtt ttaataaacc aattcggttg 660
gctatacccc ccggttaact cgtgacgttg accaatgttc gcgacgtgat cgccagcttg 720
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<210> 8
<211> 252
<212> PRT
<213> Viscum album

<400> 8

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Tyr Glu Arg Ile Arg Leu Arg Val Thr His Gln Thr Thr Gly Glu Glu
1 5 10 15
Tyr Phe Arg Phe Ile Thr Leu Leu Arg Asp Tyr Val Ser Ser Gly Ser
20 25 30
Phe Ser Asn Glu Ile Pro Leu Leu Arg Gln Ser Thr Ile Pro Val Ser
35 40 45
Asp Ala Gln Arg Phe Val Leu Val Glu Leu Thr Asn Gln Gly Gly Asp
50 55 60
Ser Ile Thr Ala Ala Ile Asp Val Thr Asn Leu Tyr Val Val Ala Tyr
65 70 75 80
Gln Ala Gly Asp Gln Ser Tyr Phe Leu Arg Asp Ala Pro Arg Gly Ala
85 90 95
Glu Thr His Leu Phe Thr Gly Thr Thr Arg Ser Ser Leu Pro Phe Asn
100 105 110
Gly Ser Tyr Pro Asp Leu Glu Arg Tyr Ala Gly His Arg Asp Gln Ile
115 120 125
Pro Leu Gly Ile Asp Gln Leu Ile Gln Ser Val Thr Ala Leu Arg Phe
130 135 140
Pro Gly Gly Ser Thr Arg Thr Gln Ala Arg Ser Ile Leu Ile Leu Ile
145 150 155 160
Gln Met Ile Ser Glu Ala Ala Arg Phe Asn Pro Ile Leu Trp Arg Ala
165 170 175
Arg Gln Tyr Ile Asn Ser Gly Ala Ser Phe Leu Pro Asp Val Tyr Met
180 185 190
Leu Glu Leu Glu Thr Ser Trp Gly Gln Gln Ser Thr Gln Val Gln His
195 200 205
Ser Thr Asp Gly Val Phe Asn Asn Pro Ile Arg Leu Ala Ile Pro Pro
210 215 220
Gly Asn Phe Val Thr Leu Thr Asn Val Arg Asp Val Ile Ala Ser Leu
225 230 235 240
Ala Ile Met Leu Phe Val Cys Gly Glu Arg Pro Ser
245 250

<210> 9
<211> 789
<212> DNA
<213> Viscum album

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aatggcagct gcttgaccac gtatggctat actgctggcg tctatgtgat gatcttcgac 240
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aatccaagat ccaatctggt tttggcagca tcatctggaa tcaaaggcac tacgcttacg 360
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cgcgaggtga ccatatatgg gttcagggac ctttgcattg aatcaaattg agggagtgtg 480
tgggtggaga cgtgctgtgag tagccaaaag aaccaaagat gggctttgta cggggatggg 540
tctatacgcc ccaaacaaaa ccaagaccaa tgcctcacct gtgggagaga ctccgtttca 600
acagtaatca atatatgttag ctgcagcgct ggatcgcttg ggcagcgatg ggtgtttacc 660
aatgaagggg ccattttgaa tttaaagaat ggggttgcca tggatgtggc gcaagcaaat 720
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ccgtgcca 789

<210> 10
<211> 263
<212> PRT
<213> Viscum album

<400> 10
Asp Asp Val Thr Cys Ser Ala Ser Glu Pro Thr Val Arg Ile Val Gly
1 5 10 15

Arg Asn Gly Met Cys Val Asp Val Arg Asp Asp Asp Phe Arg Asp Gly
20 25 30

Asn Gln Ile Gln Leu Trp Pro Ser Lys Ser Asn Asn Asp Pro Asn Gln
35 40 45

Leu Trp Thr Ile Lys Arg Asp Gly Thr Ile Arg Ser Asn Gly Ser Cys
50 55 60

Leu Thr Thr Tyr Gly Tyr Thr Ala Gly Val Tyr Val Met Ile Phe Asp
65 70 75 80

Cys Asn Thr Ala Val Arg Glu Ala Thr Leu Trp Gln Ile Trp Gly Asn
85 90 95

Gly Thr Ile Ile Asn Pro Arg Ser Asn Leu Val Leu Ala Ala Ser Ser
100 105 110

Gly Ile Lys Gly Thr Thr Leu Thr Val Gln Thr Leu Asp Tyr Thr Leu
115 120 125

Gly Gln Gly Trp Leu Ala Gly Asn Asp Thr Ala Pro Arg Glu Val Thr
130 135 140

Ile Tyr Gly Phe Arg Asp Leu Cys Met Glu Ser Asn Gly Gly Ser Val
145 150 155 160

Trp Val Glu Thr Cys Val Ser Ser Gln Lys Asn Gln Arg Trp Ala Leu
165 170 175

Tyr Gly Asp Gly Ser Ile Arg Pro Lys Gln Asn Gln Asp Gln Cys Leu
180 185 190

Thr Cys Gly Arg Asp Ser Val Ser Thr Val Ile Asn Ile Val Ser Cys

E1

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195 200 205
Ser Ala Gly Ser Ser Gly Gln Arg Trp Val Phe Thr Asn Glu Gly Ala
210 215 220
Ile Leu Asn Leu Lys Asn Gly Leu Ala Met Asp Val Ala Gln Ala Asn
225 230 235 240
Pro Lys Leu Arg Arg Ile Ile Ile Tyr Pro Ala Thr Gly Lys Pro Asn
245 250 255
Gln Met Trp Leu Pro Val Pro
260

<210> 11
<211> 48
<212> DNA
<213> Viscum album

<400> 11
tcctctgagg tgcgctattg gccgctgggc atacgaccgc tgatagcc 48

<210> 12
<211> 16
<212> PRT
<213> Viscum album

E1
<400> 12
Ser Ser Glu Val Arg Tyr Trp Pro Leu Val Ile Arg Pro Val Ile Ala
1 5 10 15

<210> 13
<211> 94
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Synthetic gene
encoding amino acids 53-78 of human P2 protein

<400> 13
gtaccgggtg gcggctgtac cgaatccacc ttcaaaaaca ccgaaatctc cttcaaactg 60
ggtcaggaat tcgaagaaac caccgctgac aact 94

<210> 14
<211> 26
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Amino acids
53-78 of human P2 protein

<400> 14
 Arg Thr Glu Ser Thr Phe Lys Asn Thr Glu Ile Ser Phe Lys Leu Gly
 1 5 10 15

Gln Glu Phe Glu Glu Thr Thr Ala Asp Asn
 20 25

<210> 15
 <211> 75
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:Fig. 20:
 Synthetic linker cassette for providing modularity
 at the 3' end of rMLB delta 1alpha 1beta

<400> 15
 caccggtaaa ccgaaccaga tgtggctgcc ggtaccgtag taacgctcct cgtcgaccta 60
 gtaaggatcc ctgga 75

<210> 16
 <211> 12
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:Fig. 20: amino
 acid sequence encoded by portion of SEQ ID NO: 15

<400> 16
 Thr Gly Lys Pro Asn Gln Met Trp Leu Pro Val Pro
 1 5 10

<210> 17
 <211> 82
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:Fig. 21:
 Synthetic linker cassette for providing modularity
 at the 3'end of rMLB Delta 1alpha 1beta 2gamma
 with affinity module ("His-Tag").

<400> 17
 ccggtaaacc gaaccagatg tggctgccgg taccgggtgg tggatatcat caccaccatc 60
 accactagta actcctcgga tc 82

<210> 18
 <211> 21
 <212> PRT
 <213> Artificial Sequence

E1

<220>

<223> Description of Artificial Sequence:Amino acid
sequence encoded by a portion of SEQ ID NO: 17

<400> 18

Gly	Lys	Pro	Asn	Gln	Met	Trp	Leu	Pro	Val	Pro	Gly	Gly	Gly	Tyr	His
1				5				10						15	

His	His	His	His	His
				20

<210> 19

<211> 26

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Codon exchange
rMLB D23A

<400> 19

catgtgcgtg gccgtccgag atgacg

26

<210> 20

<211> 27

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Fig. 22:
Mutagenic oligonucleotides for inactivating
carbohydrate binding sites in rMLB. - 1alpha2
(W38A). -

<400> 20

cagatacagt tggcgccctc caagtcc

27

<210> 21

<211> 61

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Fig. 22:
Mutagenic oligonucleotides for inactivating
carbohydrate binding sites in rMLB. - 1beta (Y68S,
Y70S, Y75S, F79S). -

<400> 21

gctgcttgac	cacgtctggc	tctactgctg	gcgtctctgt	gatgatctcc	gactgtaata	60
c						61

E1

<210> 22
 <211> 26
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:Fig. 22:
 Mutagenic oligonucleotides for inactivating
 carbohydrate binding sites in rMLB. - 1beta1
 (D235A). -

<400> 22
 gggttgcca tggctgtggc gcaagc

26

<210> 23
 <211> 26
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:Fig. 22
 Mutagenic oligonucleotides for inactivating
 carbohydrate binding sites in rMLB. - 2gamma2
 (Y249A). -

<400> 23
 cgaataatca tcgctcctgc cacagg

26

<210> 24
 <211> 35
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:Fig. 22:
 Mutagenic oligonucleotides for inactivating
 carbohydrate binding sites in rMLB. - pT7 EcoRV to
 SspI. -

<400> 24
 cttccttttt caatattatt gaagcattta tcagg

35

<210> 25
 <211> 35
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:Fig. 22:
 Mutagenic oligonucleotides for inactivating
 carbohydrate binding sites in rMLB. - pT7 SspI to
 EcoRV. -

<400> 25

E1

cttccttttt cgatatcatt gaagcattta tcagg

35

<210> 26

<211> 40

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Fig. 23:
Mutagenic oligonucleotides for constructing
modular ITF gene cassettes. - pT7 Delta NdeI to
StuI. -

<400> 26

ctttaagaag gagatataca ggcctacgag aggctaagac

40

<210> 27

<211> 33

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Fig. 23:
Mutagenic oligonucleotides for constructing
modular ITF gene cassettes. - rMLB silent NheI. -

<400> 27

gttacctgca gtgctagcga acctacggtg cgg

33

<210> 28

<211> 32

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Fig. 23:
Mutagenic oligonucleotides for constructing
modular ITF gene cassettes. - rMLA Delta AgeI. -

<400> 28

cccaccagac caccggcgaa gaatatttcc gg

32

<210> 29

<211> 40

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Fig. 23:
Mutagenic oligonucleotides for constructing
modular ITF gene cassettes.

<400> 29

E1

gtttgtatgc ggagagcgtc cctcgagctc tgaggtgcgc

40

<210> 30

<211> 43

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Fig. 23:
Mutagenic oligonucleotides for constructing
modular ITF gene cassettes. - rMLB Delta EcoNI to
AgeI. -

<400> 30

ccgaataatc atcgctccgg ccaccggtaa accaaatcaa atg

43

<210> 31

<211> 11

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Flanking region
of the ProML gene cassette in expression vector
pT7ProML

<400> 31

tacatatgta c

11

<210> 32

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Flanking region
of the ProML gene cassette in expression vector
pT7ProML

<400> 32

ccatgataag gatacctctag

20

<210> 33

<211> 9

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Flanking region
of the IML gene cassette in expression vector
PIML-02-P

<400> 33

caggcctac

9

<210> 34

<211> 34

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Flanking region
of the IML gene cassette in expression vector
PIML-02-P

<400> 34

cactagtaac tcctcggatc ctctagagtc gacc

34

<210> 35

<211> 4

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Modulator
module peptide

<400> 35

Lys Asp Glu Leu

1

<210> 36

<211> 4

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Modulator
module peptide

<400> 36

His Asp Glu Leu

1

<210> 37

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Portion of the
ML propeptide

<400> 37

Ser Ser Ser Glu Val Arg Tyr Trp Pro Leu Val Ile Arg Pro Val Ile

1

5

10

15

E1

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Ala

<210> 38

<211> 13

<212> PRT

<213> Artificial Sequence

E1
<220>

<223> Description of Artificial Sequence: A degradation
product of myelin basic protein.

<400> 38

Val His Phe Phe Lys Asn Ile Val Thr Pro Arg Thr Pro
1 5 10
